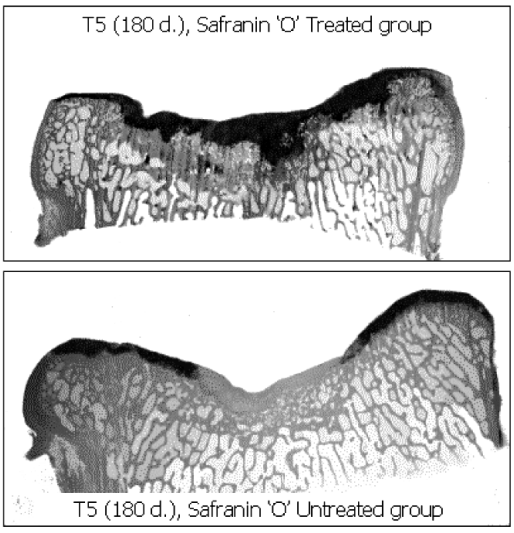

At T1,2,3 it has been possible to highlight an increase in chondrocytes proliferation and a decrease of inflammatory factors (IL-1β, MMP-9). On the contrary the Untreated group has showed a severe tissue inflammation.
At T4 and T5 we observed the formation of a new tissue with some features of hyaline-like cartilage either for the presence of type II collagen expression and for the morphological appearance. We could observe the spatial distribution of collagen fibers according to Benningoff's scheme.
Conclusions: Our preliminary data suggest that HILT could be able to induce a physiological repair of osteochondral defects. In fact the quality of the newly formed tissue was excellent in terms of shape and morphological distribution of isogenic groups, type and spatial distribution of collagen fibers and physical-chemical composition of ECM.
Moreover in the Treated group we can observe a centripetal re-growth of cartilage with higher activation of the medial edge than the lateral one, maybe due to its lower distance from the skin and therefore reached by higher intensity of light.
Future direction would be to evaluate potential use of the HILT as a non-invasive treatment for isolated chondral lesions and osteoarthritis.

A22

THE RELATIONSHIP BETWEEN SUBCHONDRAL BONE AND CARTILAGE VOLUME IN A NON-HUMAN PRIMATE MODEL OF OSTEOARTHRITIS

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Purpose: To determine the utility of high resolution computer tomography, magnetic resonance imaging, and serum biomarkers in the early detection and longitudinal progression of knee osteoarthritis in a non-human primate disease model.
OA develops spontaneously in rhesus monkeys, and the study of medial compartment disease in the species has provided some models for studying naturally occurring OA. Both the prevalence and severity of knee OA increase with age in this species.
Because the etiology of OA is poorly understood and the disease progresses slowly until significant joint impairment has occurred, designing human studies to understand the pathophysiology of the disease has been almost impossible. The use of murine and canine animal models to study OA, although useful, has generated much skepticism as to how well these models can truly mimic the disease in humans.
The rhesus macaque is the most suitable animal model for comparative studies of OA because it: 1) has a close biological relationship to humans; 2) is bipedal; and 3) spontaneously develops age-related OA in a similar age distribution to the disease found in humans.
Methods: Specimens were gathered through the National Institutes of Health Tissue Bank. All specimens were collected post-mortem and the excised limbs with intact joint capsule were stored in buffered formalin.
The fixed joints were imaged using an Imtek MicroCat-II x-ray computed tomography (CT) system. Segmentation of the images was obtained to determine the bone mineral density. The micro-MRI scanning was done on a 4.7 Tesla Oxford Instruments 40 cm horizontal bore superconducting magnet. All images were analyzed using t Amira 3-D visualization software.Blood-based RNA expression was used to correlate biomarkers with the structural changes found in the joints.
Results: The findings reported are from the analysis of 36 knee joints from animals aged 4 years, 8 years, 14 years, 25 years, 36 years and 45 years.
The micro-CT images reveal a progressive decrease in mineralization of the subchondral bone, and disintegration of the normal bony architecture at the articular surfaces of both the tibial plateau and femoral condyles in the aging rhesus macaques. These images also confirmed marked extra-articular ossification and osteophytosis.
The corresponding micro-MRI images confirmed the association of cartilage loss in the weight-bearing regions of the joint and defined areas of soft tissue alteration and degeneration in and around the joint space. These images were most valuable in the measurementof cartilage volume loss as it relates to the bone remodeling process in progressive OA.
Changes in the expression of several serum biomarkers (COMP, Osteocalcin, Alkaline Phosphatase and Hyaluronic acid) were associated with bone and cartilage degeneration imaged in the knee joints.
Conclusions: These results indicate that micro-CT, Micro-MRI and serum biomarkers, together can be used in the early detection of subchondral changes in age-related degenerative arthritis in rhesus macaques. These methods are also invaluable in longitudinal studies of the role of the subchondral bone in OA disease progression and the relationship of OA to bone mineral density.

A23

MESENCHYMAL CELLS/POLYLACTIC ACID SCAFFOLD CONSTRUCT FOR THE REPAIR OF OSTEOCHONDRAL LESIONS

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Purpose: The aim of this study was to elucidate whether mesenchymal cells (MC) could survive and proliferate in polylactic acid (PLA) scaffold in vitro and in vivo.
Methods: Bone marrow cells, isolated from the femurs and tibias of mature New Zealand White rabbits (9-12 months old), were cultured for 14 days. Fibroblast-like adhered MC which contain mesenchymal stem cells were obtained. A cylindrical biodegradable D, D-L, L-PLA core (DRILAC CUBE, Kensey Nash Co., PA) 3.7 mm in diameter and 3.0 mm in depth, and 0.5ml medium including 1x106 MC were put into a 20 ml glass scintillation vial, and were rotated at 100 rpm for 2 hours to create MC/PLA construct. After cultured for 7 days, the construct was transplanted into osteochondral defect in the medial femoral condyle. The MC behavior was analyzed as cartilage matrix formation and cellular

viability using a fluorescent in situ double-staining technique. In order to identify the number of MC, male-derived MC with PLA were transplanted into osteochondral defects of female femurs. The male-derived sex-determining region Y (SRY) gene was used as a marker of MC detecting by polymerase chain reaction (PCR), and matrix metalloproteinase-1 (MMP-1), which exists in male and female autosomal gene, was used as a control gene. The total cell and MC number were calculated using NIH imaging software as a quantitative method.

Results: MC survived in the PLA (Fig. 1), and produced a matrix of collagen fibers (Fig. 2) and glycosaminoglycans (Fig. 3) at 1 week after culturing in vitro. Following that, at 1 week after the transplantation of the construct, 79% of total cells in the defect were MC. However, the ratio of MC decreased with time, and finally, transplanted cells were not detected in the defects at 24 weeks (Fig. 4).

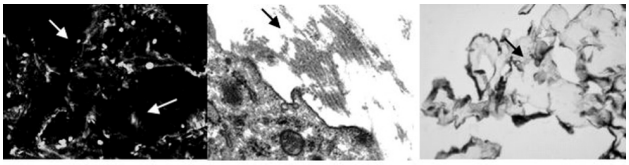

Fig. 1 Fig. 2 Fig. 3

Fig. 1. Arrows indicate cellular viability of MC in PLA assessed by confocal laser scanning microscopy.
Fig. 2 Presence of glycosaminoglycan stained with safranin O.
Fig. 3. Presence of collagen fiber detected by electron microscope.

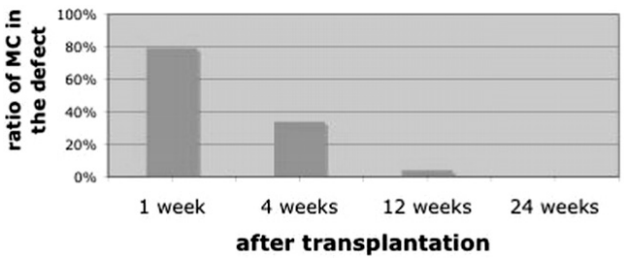

Fig. 4. Variable of MC ratio in the defect with time in vitro.

Conclusions: MC attached and produced cartilage matrix in the PLA in vitro. In vivo condition, MC could survive in osteochondral defect, and then were replaced by host cells within 24weeks. This study to evaluate the survivability of transplanted MC in vivo is essential for further tissue engineering strategy to enhance cartilage regeneration in osteochondral defect.

A24

MURINE SURGICALLY-INDUCED OSTEOARTHRITIS IS SEX AND STRAIN DEPENDENT

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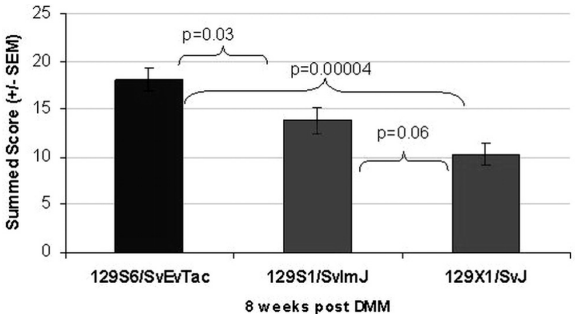
Purpose: Many inbred strains of mice exist and are used for specific disease models. Evaluation of wild-type mice of different genetic background has found huge variability in susceptibility to OA following surgical destabilization of the medial meniscus (DMM). 129SvEv, C57BL6 and C57BL10 mice have more OA than FVB/n, which has more OA than DBA/1J. Sex differences are also observed in this model, with females having less OA than males. To extend upon these observations, 3 different 129 strains (commonly in use for knock out (KO) creation), as well as castrated mice (with and without testosterone replacement), were evaluated in the DMM model.

Methods: All animal studies were performed in accord with Wyeth IACUC protocols. Three 129Sv strains (129S6/SvEvTac, 129S1/SvImJ and 129X1/SvJ) underwent DMM surgery at 10 weeks of age with 20 mice/group. Intact and castrated 129S6/SvEvTac mice were purchased from Taconic and underwent the DMM surgery as well as addition of testosterone or placebo slow-release pellets, implanted subcutaneously. Following sacrifice at 8 weeks post-operatively, the knees were decalcified, stained with Safranin-O/Fast green and scored blindly by 2 observers using a semi-quantitative system in which a higher score reflects greater OA severity.

Results: 129X1/SvJ had far less OA than 129S6/SvEvTac. The scores of 129S1/SvImJ were intermediate. Castration of male mice resulted in significantly less OA (compared to intact males), which was reversible with the addition of testosterone.



Conclusions: Susceptibility to OA varied dramatically across different murine strains, including different 129 strains. Distinguishing exactly which background a KO mouse is created and back-crossed onto is critical for accurate interpretation of any OA KO studies. In addition, intact male mice have more OA than female mice or castrated male mice, and addition of testosterone to castrated males increases OA severity. Further studies are needed to assess if testosterone enhances activity levels, or has adverse effects on musculoskeletal and/or cartilage properties.

A25

COLLAGEN TYPE II DEGRADATION AND FORMATION ASSESSED IN EX VIVO AND IN VIVO IN MODELS OF RA

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Purpose: Cartilage erosion in rheumatoid arthritis (RA) is a result of over-expression of pro-inflammatory cytokines that leads to protease expression and cartilage destruction. We investigated whether collagen type II degradation and formation in serum and locally in the joint would reflect disease status in an animal model of RA. Furthermore, we used an *ex vivo* model of articular cartilage degradation to assess the direct effect of pro-inflammatory cytokines on collagen type II turnover.

Methods: *In vivo:* Arthritis was induced in 10 female Lewis rats (150-175 g) by intradermal injection of porcine type II collagen at the base of the tail at day 0 and 7. Hind paw score (0-4, 4=most severe) and hind paw volume was measured from day 8 after immunization. Serum samples was collected at baseline and day 7, onset+1 day, onset+7 days and onset+14 days, where after the animals were terminated. One hind paw was snap-frozen in nitrogen, homogenized and extracted for proteins, while the other hind paw was fixed in formaldehyde and decalcified in EDTA for histology and immunohistochemistry.*Ex vivo*: Bovine articular cartilage explants were cultured in presence of oncostatin M (OSM) 10 ng/mL, tumor necrosis factor-α (TNF) 20 ng/mL for